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A Two-Year Surveillance in Five Colombian Tertiary Care Hospitals Reveals High Frequency of Non-CG258 Clones of Carbapenem-Resistant *Klebsiella pneumoniae* with Distinct Clinical Characteristics

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The global spread of carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) has been largely associated with sequence type 258 (ST258) and its related variants (clonal group 258 [CG258]). Here we describe the molecular epidemiology of CR-Kp from five tertiary care hospitals in Medellín, the second largest city in Colombia. All CR-Kp-infected patients admitted from June 2012 to June 2014 were included ($n = 193$). Patients' clinical information was obtained from medical records. Carbapenemase KPC, VIM, IMP, NDM, and OXA-48 genes were detected by PCR. A CG258-*tonB79* cluster-specific real-time PCR (targeting the multilocus sequence type [MLST] *tonB79* allele), pulsed-field gel electrophoresis (PFGE), and MLST analysis were performed for typing. Remarkably, 62.2% ($n = 120$) of isolates were from STs unrelated to CG258 (non-CG258). KPC-3 predominated in CG258 isolates (86.3%), while KPC-2 prevailed in non-CG258 isolates (75.5%) ($P < 0.001$). Multidrug resistance (MDR) frequency was significantly higher in CG258 strains (91.4% versus 56.1%; $P < 0.001$). ST512 (a single-locus variant of ST258) is the main ST in CG258 (96.3%), and isolates in this group showed closely related pulsotype and similar resistance gene profiles, suggesting the clonal spread of this strain. In contrast, high heterogeneity of STs (34/54), including eight novel STs, was found in non-CG258 isolates. Among non-CG258 isolates, ST14 (13.3%; $n = 16$) and ST307 (14.2%; $n = 17$) were the most frequent, and they showed distinct molecular and clinical characteristics in comparison to CG258 isolates. Our results suggest that the dissemination of carbapenem resistance in Medellín is due to heterogeneous *K. pneumoniae* clones, likely the result of horizontal transmission of KPC in different unrelated lineages, further highlighting the challenge in CR-Kp infection control and the need for a multifocal intervention.

Carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) is currently one of the most important pathogens causing health care-associated infections, which typically occur in patients with prolonged hospital stay and previous antibiotic exposure (1–3). The most common mechanism of carbapenem resistance in *K. pneumoniae* is the production of carbapenemases, among which *K. pneumoniae* carbapenemase (KPC) is by far the most relevant and prevalent (4, 5).

Since the initial identification of KPC in 1996 in North Carolina (6), KPC-producing strains have spread worldwide, causing major hospital outbreaks in North America, Europe, Asia, and Latin America (2, 7, 8) and leading to a reduction of therapeutic options that has resulted in high mortality and morbidity rates and increasing length of hospital stay and associated costs (2, 4, 5, 9). The global spread of KPC-producing *Klebsiella pneumoniae* (KPC-Kp) has been linked mostly to one genetic lineage, the multilocus sequence type 258 (ST258) and its related variants, i.e., clonal group 258 (CG258) (1, 10, 11). Members of CG258 include ST258, its single-locus variants (SLVs) (e.g., ST11, ST512, ST340, and ST437), and their SLVs (e.g., ST650 [an SLV to ST512]) (1). CG258 has been associated with 70% of KPC-Kp outbreaks in the United States and about 90% of infections by KPC-Kp in Israel, and it is the most predominant clone in Argentina and Brazil (5).

In South America, the first report of KPC-2-producing *K. pneumoniae* infections came in 2005, based on cases in two patients from two different hospitals in Medellín, Colombia (12). Furthermore, an outbreak at an intensive care unit (ICU) occurred between 2007 and 2008 in the same city, where the index case involved a patient who had traveled from Israel. The outbreak was due to KPC-3-producing *K. pneumoniae* strains typed as ST512, an SLV to ST258 and a member of CG258 (13, 14). Further studies in Colombia have shown the increasing incidence of KPC producers among other *Enterobacteriaceae*, including *Klebsiella oxytoca*, *Enterobacter cloacae*, *Serratia marcescens*, and *Escherichia coli* (14–16), and other Gram-negative bacilli, such as *Pseudomonas aeruginosa* (16–19). Curiously, in these non-*K. pneumoniae* isolates, KPC-2 was the predominant variant, while KPC-3 was frequently found in *K. pneumoniae*, especially isolates from CG258 (16–19).

Results from the SENTRY Antimicrobial Surveillance Program showed that the frequencies of CR-Kp in Brazil, Argentina, and Chile were 11.1%, 8.2%, and 5.0%, respectively (20). In contrast,

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the frequency of CR-Kp in Colombia is as high as 14% based on the data from the health care-associated infections and antimicrobial resistance national surveillance system of the Colombian National Institute of Health (INS) (21). Colombia is now regarded as one region where CR-Kp is endemic (5). Currently, knowledge of the clinical, epidemiological, and molecular features of the circulating KPC-Kp strains is still limited. A better understanding of the epidemiology of KPC-Kp infections in countries where KPC strains are endemic, such as Colombia (5), is crucial in identifying the factors contributing to the dissemination of KPC-Kp and is essential in developing targeted strategies for prevention and infection control, both regionally and globally (22, 23). Here we describe a large cross-sectional study aimed at exploring the molecular epidemiology of CR-Kp from five tertiary care hospitals in Medellín, Colombia, from 2012 to 2014.

MATERIALS AND METHODS

Study population and settings. This cross-sectional study included all inpatients with carbapenem-nonsusceptible infections admitted from June 2012 to June 2014 at five referral tertiary care hospitals (providing service to both pediatric and adult patients) in Medellín, the second largest city in Colombia. Hospitals A and C are large university hospitals, with 754 and 700 beds, respectively. Hospitals B and D are medium-size tertiary care centers, with 286 and 300 beds, respectively. Hospital E is a 140-bed cardiology hospital. The study protocol was approved by the Bioethics Committee for Human Research at Universidad de Antioquia (CBE-SIU) (approval no. 11-35-415), as well as by the research ethics committees at each hospital.

Clinical and epidemiological information. Medical records were reviewed for each patient. Clinical data, including demographics, medical history, comorbidities, treatment, and outcome at discharge, were collected. Infections were classified as either community or health care associated according to standard epidemiological definitions established by the U.S. Centers for Disease Control and Prevention (CDC) (24).

Bacterial strains and antibiotic susceptibility. In this study, CR-Kp was defined as carbapenem nonsusceptible (intermediate or resistant) based on the CLSI 2015 guidelines (25). Nonduplicate carbapenem-nonsusceptible *K. pneumoniae* isolates were selected. The identification of isolates as well as the antibiotic susceptibility testing was carried out on the automated system Vitek 2 (bioMérieux, Marcy l'Etoile, France). Tested antibiotics included extended-spectrum cephalosporins (ceftriaxone, ceftazidime, and cefepime), carbapenems (ertapenem, imipenem, and meropenem), aminoglycosides (amikacin and gentamicin), quinolones (ciprofloxacin), tigecycline, and colistin. Antibiotic resistance to colistin and tigecycline was further confirmed by microdilution method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) and FDA breakpoints, respectively.

Detection of β-lactamases. The presence of carbapenemases was evaluated by the modified Hodge test (MHT), performed according to the CLSI protocol (25). bla_{KPC} variants were detected using a molecular beacon-based multiplex real-time PCR assay (7). Conventional multiplex PCR assays were performed for carbapenemase genes, i.e., bla_{VIM}, bla_{IMP}, bla_{NDM}, and bla_{OXA-48} (26, 27); extended-spectrum-β-lactamase (ESBL) genes, i.e., bla_{CTX-M} (clusters 1, 2, 8, 9, and 25), bla_{SHV}, and bla_{TEM}; and genes for plasmid-mediated AmpC β-lactamases, including $bla_{ACT/MIR}$, $bla_{CMY-1/MOX}$, $bla_{CMY-2/LAT}$, bla_{FOX}, bla_{DHA}, and bla_{ACC} (28). All PCRs were performed using previously validated primers and conditions (6, 26, 27). For β-lactamase variants, amplification products were sequenced in a subset of isolates, and the nucleotide and deduced amino acid sequences of *bla* genes were compared against reference sequences available in the NCBI (National Center for Biotechnology Information) GenBank database using the BLAST (Basic Local Alignment Search Tool) server and MultAlin software for sequence alignment (http://multalin.toulouse.inra.fr/multalin/).

Phylogenetic typing. All isolates were tested by a previously validated CG258-*tonB79* cluster-specific multiplex real-time PCR (29). This PCR was designed to target the multilocus sequence type (MLST) *tonB79* allele, which is primarily found in ST258 and its single-locus (SLV) and double-locus (DLV) variants, e.g., ST512 (1, 30). A polysaccharide synthesis gene cluster (CPS) multiplex PCR (31) was then performed on all CG258-*tonB79* isolates to identify the ST258 sublineages (clades I and II). Pulsed-field gel electrophoresis (PFGE) was performed on all non-CG258-*tonB79* isolates and among a subset of CG258-*tonB79* isolates (40%) as described previously (32). Strain relatedness analysis was performed on BioNumerics software version 6.0 (Applied Maths, Sint-Martens-Latem, Belgium) based on the Dice coefficient (cutoff of 80 or higher) for genetic relatedness and the unweighted-pair group method analysis using average linkages (UPGMA) for generation of dendrograms. DNA fragment patterns were normalized using a bacteriophage lambda ladder PFGE marker (New England BioLabs, United Kingdom) with a 1% position tolerance for further analysis. Representative isolates with unique PFGE patterns ($n = 54$; 28%) were further subject to MLST analysis using the protocol described by Diancourt et al. (33).

Statistical analyses. To explore the association between clinical and epidemiological characteristics of infected patients and strain genotypes, the chi-square, Fisher's exact, or Kruskal-Wallis test was used. The level of statistical significance was defined as a P value of ≤ 0.05. All statistical analyses were performed in SPSS software v.22.

RESULTS

General clinical characteristics and antibiotic susceptibility. One hundred ninety-three patients infected by CR-Kp were enrolled. The patients' demographic and clinical characteristics are summarized in Table 1. The majority of the patients were males (62.7%; $n = 121$), with a median age of 54 years (interquartile [IQ] range, 35 to 70 years). Only 14.5% ($n = 28$) of patients were pediatric (aged ≤14 years). The most frequent medical specialties that provided care to patients were surgery (24.6%; $n = 47$) and internal medicine (17.8%; $n = 34$). The median hospital stay was 13 days (IQ range, 2 to 29 days). A total of 28.5% ($n = 55$) of patients were hospitalized in intensive care units (ICUs) at the time of sample collection.

The majority of patients had underlying illnesses, with cardiovascular disease (26.6%; $n = 49$), chronic renal disease (23.9%; $n = 44$), and diabetes mellitus (23.9%; $n = 44$) the most frequent. The most prevalent infections were urinary tract (33.3%; $n = 64$), bloodstream (20.3%; $n = 39$), and intra-abdominal (17.7%; $n = 34$) infections. Ninety-eight percent of infections ($n = 189$) were classified as health care associated according to CDC criteria after individual assessment. A total of 66.3% ($n = 126$) of patients had undergone surgery within a year prior to infection, 78.9% ($n = 150$) had a medical history of hospitalization within the prior 6 months, and 94.7% ($n = 180$) had a history of recent antibiotic use, mainly carbapenems (49.4%; $n = 89$) and piperacillin-tazobactam (49.4%; $n = 89$). Meanwhile, carbapenems (32.4%; $n = 47$) and piperacillin-tazobactam (22.8%; $n = 33$) were the most frequently used empirical antibiotic agents. However, with respect to targeted therapy documented for 160 patients, 40% ($n = 64$) received monotherapy, with aminoglycosides (13.8%; $n = 22$) and colistin (9.4%; $n = 15$), while carbapenem plus colistin (11.3%; $n = 18$) and aminoglycoside plus tigecycline (8.1%; $n = 13$) were the most frequently used combination therapies. At discharge, 66% ($n = 124$) of the patients had clinical improvement, and the overall mortality rate was 34% ($n = 64$); however, this varied with respect to different infection types ($P = 0.001$). The mortality rates were higher in patients with bacteremia (56.4%;

TABLE 1 Sociodemographic and clinical characteristics of carbapenem-resistant *Klebsiella pneumoniae*-infected patients from five tertiary care institutions, Medellín, Colombia

Patient characteristic	Value for total patients (n = 193)		Value for patients with:							
			CG258 isolates (n = 73; 37.8%)		Non-CG258 isolates (n = 120; 62.2%)					
					ST14 (n = 16; 8.3%)		ST307 (n = 17; 8.8%)		Other (n = 87; 45.1%)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Sociodemographic										
Age, yrs (median, IQ range)[a]	54	35–70	58	39–75	4.5	0.38–28	56	48–65	56	42–70
Male gender	121	62.7	50	68.5	8	50.0	10	58.8	53	60.9
Clinical										
Days of hospital stay (median, IQ range)	13	2–29	13	1–29	13	6–41	24	11–32	12	1–26
Hospitalization in ICU at time of isolate collection[b]	55	28.5	16	21.9	8	50.0	2	11.8	29	33.3
Health care-associated infection	189	97.9	72	98.6	16	100.0	17	100.0	84	96.6
Medical specialty or service[a]										
Surgery	47	24.6	14	19.2	1	6.3	6	35.3	26	30.6
Medicine	34	17.8	18	24.7	2	12.5	1	5.9	13	15.3
Intensive care	30	15.7	10	13.7	3	18.8	1	5.9	16	18.8
Pediatrics	22	11.5	5	6.8	8	50.0	1	5.9	8	9.4
Transplants	12	6.3	3	4.1	0	0.0	1	5.9	8	9.4
Hematology	10	5.2	7	9.6	1	6.3	2	11.8	0	0.0
Urology-nephrology	20	10.5	8	11.0	1	6.3	3	17.6	8	9.4
Orthopedics and traumatology	8	4.2	4	5.5	0	0.0	2	11.8	2	2.4
Other	8	4.2	4	5.5	0	0.0	0	0.0	4	4.7
Medical history within 1 yr prior to infection										
Colonization with CR-Kp[a]	61	31.6	33	45.2	9	56.3	3	17.6	16	18.4
Surgery	126	66.3	46	64.8	10	62.5	12	70.6	58	67.4
Medical history within 6 mo prior to infection										
Hospitalization	150	78.9	59	83.1	14	87.5	10	58.8	67	77.9
ICU stay	71	37.4	21	29.6	10	62.5	7	41.2	33	38.4
Home health	16	8.4	4	5.6	1	6.3	0	0.0	11	12.8
Dialysis	45	23.7	18	25.4	3	18.8	6	35.3	18	20.9
Immunosuppressive conditions or therapies	53	27.9	21	29.6	6	37.5	5	29.4	21	24.4
Chemotherapy	22	11.6	12	16.9	3	18.8	0	0.0	7	8.1
Antibiotic use within 6 mo prior to infection	180	94.7	68	95.8	16	100.0	17	100.0	79	91.9
Piperacillin-tazobactam	89	49.4	34	50.0	7	43.8	6	35.3	42	53.2
Carbapenems	89	49.4	33	48.5	7	43.8	8	47.1	41	51.9
Aminoglycosides[a]	33	18.3	16	23.5	3	18.8	8	47.1	6	7.6
Fluoroquinolones	57	29.5	25	34.2	6	37.5	5	29.4	21	24.1
Underlying conditions	184	100.0	69	100.0	16	100.0	17	100.0	82	100.0
Cancer	33	17.9	11	15.9	2	12.5	3	17.6	17	20.7
Diabetes mellitus[b]	44	23.9	19	27.5	2	12.5	8	47.1	15	18.3
Chronic renal disease	44	23.9	18	26.1	2	12.5	6	35.3	18	22.0
Cardiovascular disease[b]	49	26.6	10	14.5	5	31.3	6	35.3	28	34.1
Sites of infection[b]										
Pneumonia	13	6.8	2	2.7	2	12.5	1	5.9	8	9.3
Bloodstream	39	20.3	19	26.0	5	31.3	6	35.3	9	10.5
Surgical site	17	8.9	6	8.2	0	0.0	2	11.8	9	10.5
Skin and soft tissue	10	5.2	3	4.1	0	0.0	4	23.5	3	3.5
Intra-abdominal	34	17.7	9	12.3	2	12.5	1	5.9	22	25.6
UTI	64	33.3	30	41.1	5	31.3	2	11.8	27	31.4
Osteomyelitis	5	2.6	2	2.7	1	6.3	0	0.0	2	2.3
Other	10	5.2	2	2.7	1	6.3	1	5.9	6	7.0
Invasive medical devices at time of infection	141	100.0	54	100.0	12	100.0	12	100.0	63	100.0
Urinary catheter	87	61.7	35	64.8	6	50.0	10	83.3	36	57.1
Vascular dialysis catheter[b]	25	17.7	13	24.1	0	0.0	6	50.0	6	9.5
Invasive mechanical ventilation	35	24.8	11	20.4	4	33.3	3	25.0	17	27.0
Enteral nutrition	53	37.6	18	33.3	7	58.3	4	33.3	24	38.1
Central venous catheter	83	58.9	33	61.1	9	75.0	7	58.3	34	54.0

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TABLE 1 (Continued)

Patient characteristic	Value for total patients (n = 193)		Value for patients with:							
			CG258 isolates (n = 73; 37.8%)		Non-CG258 isolates (n = 120; 62.2%)					
					ST14 (n = 16; 8.3%)		ST307 (n = 17; 8.8%)		Other (n = 87; 45.1%)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Empirical therapy	145		49		14		12		70	
Aminoglycoside	9	6.2	5	10.2	2	14.3	0	0	2	2.9
Carbapenem	47	32.4	15	30.6	3	21.4	6	50	23	32.9
Carbapenem + aminoglycoside	6	4.1	3	6.1	1	7.1	0	0	2	2.9
Carbapenem + colistin	5	3.4	2	4.1	0	0	0	0	3	4.3
Carbapenem + TMP-SMX[c]	3	2.1	0	0	1	7.1	1	8.3	1	1.4
Cefepime	4	2.8	1	2	0	0	0	0	3	4.3
Colistin	4	2.8	4	8.2	0	0	0	0	0	0
Fluoroquinolone + colistin	3	2.1	0	0	1	7.1	1	8.3	1	1.4
Piperacillin-tazobactam	33	22.8	10	20.4	2	14.3	2	16.7	19	27.1
Piperacillin-tazobactam + aminoglycoside	3	2.1	1	2	2	14.3	0	0	0	0
Piperacillin-tazobactam + carbapenem	5	3.4	1	2	0	0	1	8.3	3	4.3
Other	23	15.9	7	14.3	2	14.3	1	8.3	13	18.6
Targeted therapy										
Aminoglycoside	22	13.8	9	15.8	3	20	0	0	10	13.7
Aminoglycoside + fluoroquinolone	6	3.8	0	0	1	6.7	0	0	5	6.8
Aminoglycoside + tigecycline	13	8.1	7	12.3	0	0	3	20	3	4.1
Carbapenem	9	5.6	3	5.3	1	6.7	1	6.7	4	5.5
Carbapenem + aminoglycoside	7	4.4	3	5.3	0	0	0	0	4	5.5
Carbapenem + colistin	18	11.3	6	10.5	6	40	0	0	6	8.2
Carbapenem + tigecycline	4	2.5	2	3.5	0	0	0	0	2	2.7
Carbapenem + tigecycline + colistin	8	5	3	5.3	0	0	1	6.7	4	5.5
Colistin	15	9.4	6	10.5	3	20	0	0	6	8.2
Fluoroquinolone	8	5	0	0	0	0	1	6.7	7	9.6
Tigecycline	5	3.1	3	5.3	0	0	1	6.7	1	1.4
Tigecycline + colistin	4	2.5	2	3.5	0	0	1	6.7	1	1.4
Other	41	25.6	13	22.8	1	6.7	7	46.7	20	27.4
Need for surgical treatment	40	20.7	15	20.5	2	12.5	4	23.5	19	21.8
Outcome										
Death (not attributable to infection)	64	33.9	25	34.2	4	25.0	9	52.9	26	31.3
Remission or clinical improvement	124	66.0	48	65.8	12	75.0	8	47.1	56	68.3

[a] Statistically significant differences with a P value of ≤0.001 when variable was compared among CG258, ST14, ST307, and other non-CG258 isolates by Fisher's exact test or Kruskal-Wallis test.
[b] Statistically significant differences with a P value of <0.05 when variable was compared among CG258, ST14, ST307, and other non-CG258 isolates by Fisher's exact test or Kruskal-Wallis test.
[c] TMP-SMX, trimethoprim-sulfamethoxazole.

n = 22), pneumonia (50%; n = 6), and intra-abdominal infections (47.1%; n = 16) but lower in patients with urinary tract infections (UTI) (11.1%; n = 7).

Overall, high frequencies of fluoroquinolone and aminoglycoside resistance were observed, with 76% (n = 146) of isolates resistant to ciprofloxacin, 49.7% (n = 96) resistant to gentamicin, and 36.3% (n = 70) resistant to amikacin. Of clinical significance, 25.4% (n = 49) were resistant to tigecycline and 17% (n = 32) to colistin, and alarmingly, 13 isolates (7%) were resistant to both tigecycline and colistin. Moreover, 70.4% (n = 136) of strains were resistant to three or more classes of antibiotics (i.e., showed multidrug resistance [MDR]).

β-Lactamase detection. The modified Hodge test was positive for 89% (n = 173) of the isolates tested. bla_{KPC} was detected in 86% (n = 166) of the isolates, and bla_{VIM} was detected in one isolate. No OXA-48, NDM, or IMP carbapenemase genes were detected. Among KPC-positive isolates, 51.2% (n = 85) carried KPC-3, while 48.7% (n = 81) carried KPC-2; no other variants were detected. A total of 165 isolates were tested for the presence of other β-lactamases. All isolates carried SHV-11 or SHV-1 (n = 165; 100%), 45% (n = 74) of the strains harbored TEM-1, 22% (n = 37) carried CTX-M-15, and 17% (n = 28) carried SHV-12. CTX-M-2 (5%; n = 8), CTX-M-8 (2%; n = 3), TEM-11 (4%; n = 7), and SHV-27 (1%; n = 2) were also identified. AmpC β-lactamases, ACT/MIR, CMY-1/MOX, CMY-2/LAT, FOX, DHA, and ACC, were not detected in this study. Twenty-five isolates were negative for the carbapenemases, but all carried SHV-1 or SHV-11, and 20 of them harbored other β-lactamases, mostly CTX-M-15, TEM-1, and SHV-12.

Molecular typing. Among the 193 isolates, only 37.8% (n = 73) were from the CG258-*tonB79* cluster, and all exclusively carried the ST258 *cps-2* operon, belonging to ST258 clade II (11, 31). Further MLST analysis of 27 CG258-*tonB79* cluster isolates showed that 26 (96.3%) of them were ST512 and 1 was ST258. The

ST512 isolates were clustered together based on PFGE analysis (Fig. 1). One CG258 ST11 isolate was also identified by MLST analysis. Remarkably, the remaining 62.2% ($n = 120$) of isolates belonged to genetic lineages other than CG258, showing highly heterogeneous genetic backgrounds. Thirty-four different STs, i.e., ST14, -17, -23, -35, -40, -45, -129, -140, -147, -151, -231, -259, -268, -280, -283, -307, -526, -560, -636, -971, -1138, -1198, -1377, -1533, -1661, -1681, -1703, -1704, -1705, -1706, -1707, -1708, -1886, and -1887, were found. Among them, eight novel STs (ST1703 to ST1708, ST1886, and ST1887) were first identified in this study. ST14 and ST307 were the most predominant, accounting for 13.3% ($n = 16$) and 14.2% ($n = 17$) of the non-CG258 isolates, respectively. Isolates from the same STs (ST14 and ST307) were clustered together by PFGE (Dice coefficient of ≥80%) (Fig. 1).

Among KPC-positive isolates, 31 different STs were identified. CG258 accounted for 43%, while ST307 and ST14 accounted for 10% and 8%, respectively. The remaining non-CG258 strains accounted for 39% of KPC producers. Among KPC-negative strains, 8 STs were found. Interestingly, the majority of KPC-negative strains (23/26, 88%) were not related to CG258.

Comparison of clinical and molecular characteristics in different clonal groups. We compared the molecular and clinical characteristics of CG258, ST14, ST307, and other non-CG258 isolates.

(i) CG258 isolates. CG258 comprised 37.8% ($n = 73$) of the isolates. CG258 was isolated from four institutions but was the dominant clone in hospital C (75%; $n = 44$) ($P < 0.001$), followed by hospital B (35%; $n = 19$) (Fig. 2). The frequency of CG258 appeared to decrease during the study period, especially in the second half of 2013 (2013/2) ($P < 0.001$) (Fig. 3). Patients were attended most frequently by internal medicine (24.7%, $n = 18$), and 45.2% ($n = 33$) had a medical history of colonization by CR-Kp within the year prior to infection (Table 1). Isolates from CG258 were commonly multidrug resistant (94.5%; $n = 69$) and had higher frequencies of resistance to ciprofloxacin (94.5%; $n = 69$) ($P < 0.001$), amikacin (75.3%; $n = 55$) ($P < 0.001$), colistin (37%; $n = 27$) ($P < 0.001$), and tigecycline (31%; $n = 23$) than did other clonal groups. All strains except one in this group were KPC producers. Analysis of the resistance genotypes identified KPC-3 as the most frequent variant (86.3%; $n = 63$) and TEM-1 as the most common β-lactamase (56.5%), followed by the ESBL SHV-12 (19.7%).

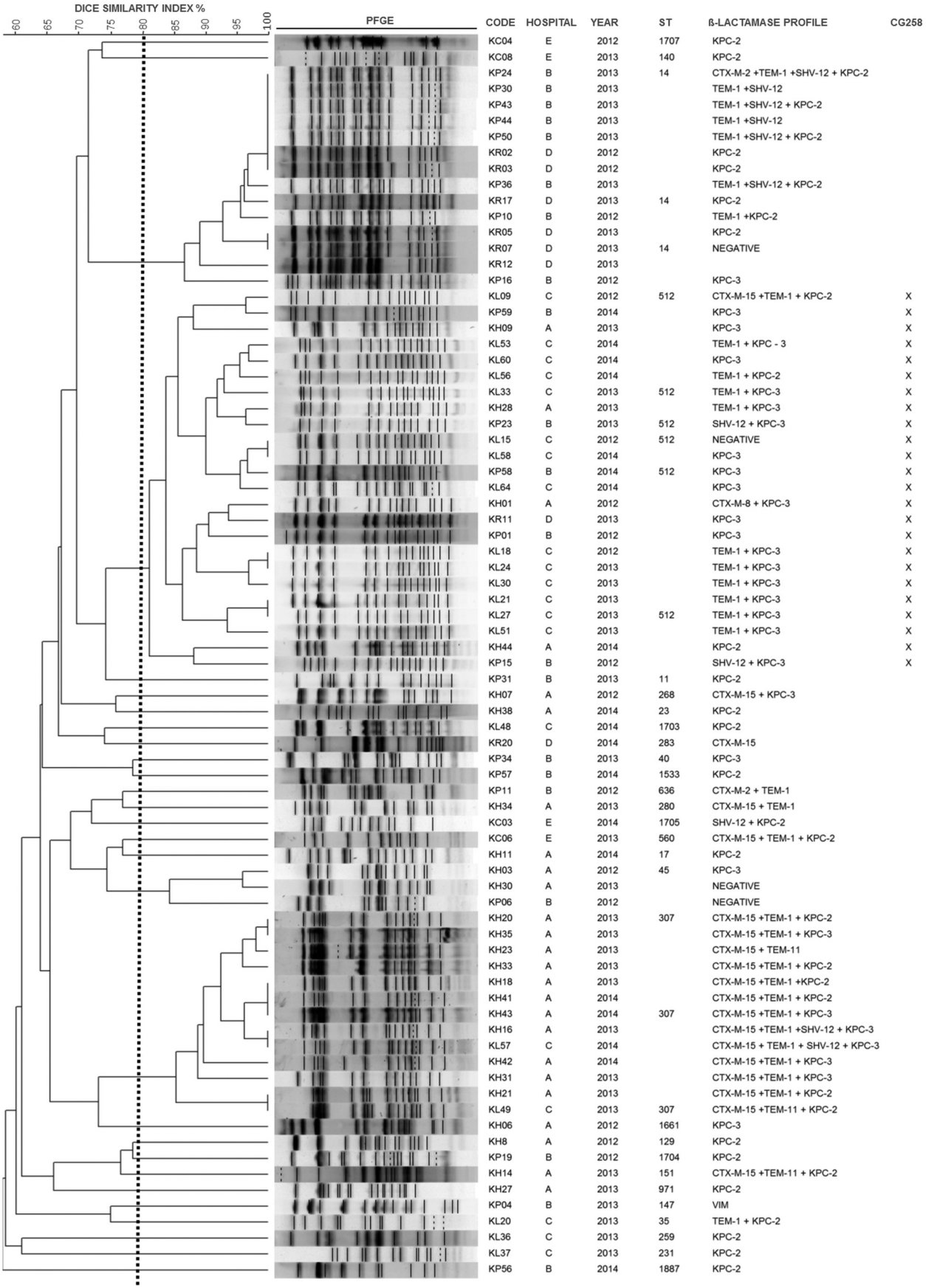
(ii) ST14 isolates. ST14 comprised 8.3% ($n = 16$) of the isolates. It was found in four institutions; however, 66% ($n = 10$) of strains were isolated from hospital B, and they accounted for 19% of that hospital's overall isolates. The frequency of ST14 isolates remained relatively constant throughout the study period, with a slight increase in the first half of 2013 (Fig. 3). Interestingly, the median age of the patients infected with the ST14 strains was 4.5 years (IQ, 0.38 to 28 years), significantly lower than the median age (52 to 58 years) of patients infected by other clonal groups (Table 1). Similarly, pediatrics was the most frequent medical specialty (50%; $n = 8$) ($P < 0.001$). Moreover, most of the patients had bloodstream infections (31.3%; $n = 5$) and UTI (31.3%; $n = 5$), half of the patients (50%; $n = 8$) were hospitalized in an ICU at the time of sample collection ($P = 0.039$), and 56.39% ($n = 9$) had a medical history of colonization by CR-Kp within the year prior to infection ($P < 0.001$) (Table 1). In addition, ST14 isolates showed higher frequencies of resistance to tigecycline (50%; $n = 8$) and gentamicin (75%; $n = 12$) than did other groups ($P < 0.001$). All isolates except two carried KPC, with KPC-2 as the most frequent variant (92.9%; $n = 13$) ($P < 0.001$). The ESBL SHV-12 was found in 7 isolates (43.8%).

(iii) ST307 isolates. ST307 comprised 8.8% ($n = 17$) of the isolates. In contrast to other clonal groups, ST307 was found only in hospitals A and C ($P < 0.001$), and the majority of the isolates (82.4%; $n = 14$) were found in hospital A, accounting for 29% of isolates from this hospital (Fig. 2). Remarkably, ST307 isolates emerged in the second half of 2013 (70% of all ST307 isolates; $n = 12$) (Fig. 3). It is noteworthy that isolates showed close patterns by PFGE (Fig. 1), suggesting the occurrence of an outbreak. More than one-third of patients were from the department of surgery ($P = 0.001$) (35.3%; $n = 6$), and 47.8% had exposure to aminoglycosides within the prior 6 months ($n = 8$) ($P = 0.001$); diabetes mellitus was the most frequent comorbidity (47%; $n = 10$) ($P = 0.024$), and skin and soft tissue infections were the most prevalent (23%; $n = 4$) ($P = 0.0013$). Although we observed no significant differences in outcomes, a higher mortality rate was found in ST307-infected patients, 52.9% ($n = 9$), than in patients infected with other clonal groups (25 to 34%) (Table 1). In addition, ST307 isolates showed higher rates of resistance to gentamicin and ciprofloxacin (88.2%; $n = 15$ each) than did other clonal groups ($P < 0.001$). All isolates except one carried KPC, with KPC-2 as the most frequent variant (62.5%; $n = 10$). The ESBL CTX-M-15 was found in 94.1% of isolates ($n = 16$).

(iv) Other non-CG258 isolates. Other non-CG258 isolates comprised 45.1% ($n = 87$) of the total isolates, including 32 different STs, and were distributed in all institutions. They accounted for the majority of isolates in hospitals A (51%), D (78%), and E (88%) and almost half in hospital B (46%), whereas they accounted for only 20% of isolates in hospital C (Fig. 2). In comparison to isolates from other groups, 22% ($n = 13$) were resistant only to β-lactams (including carbapenems) but susceptible to other antimicrobial agents. Interestingly, a higher proportion (92%; 23 of 25 carbapenemase-negative isolates) of non-carbapenemase producers were observed within this group, suggesting that additional mechanisms, e.g., the loss of porin activity, contributed to carbapenem resistance in our study. Seventy-two percent ($n = 63$) of isolates carried KPC. A total of 39% of KPC producers ($n = 27$) harbored only the KPC gene with no other β-lactamases. KPC-2 was found in 75% ($n = 48$). Moreover, the only VIM producer isolate belonged to ST147 from this group.

DISCUSSION

Transmission of KPC can be mediated by two different strategies: the horizontal transfer of KPC-harboring genetic elements (e.g., plasmid) and the clonal spread of KPC-producing strains (1). Surprisingly, highly heterogeneous genetic backgrounds were observed in KPC-Kp in this study, and 28 STs have been found to contain KPC. This suggests that KPC has emerged into different *K. pneumoniae* strains, presumably due to the horizontal transfer of bla_{KPC}-harboring plasmids. Other studies based on whole-genome sequencing analysis have described the unexpected dispersal of bla_{KPC} to many non-ST258 lineages involving different bla_{KPC} plasmids in settings where ST258 is endemic (34). Further studies are warranted to understand if the spread of KPC in multiple *K. pneumoniae* strains is due to the same plasmid or different ones. Meanwhile, three clones, CG258, ST14, and ST307, with different clinical features accounted for >50% of total isolates.



CODE	HOSPITAL	YEAR	ST	ß-LACTAMASE PROFILE	CG258
KC04	E	2012	1707	KPC-2	
KC08	E	2013	140	KPC-2	
KP24	B	2013	14	CTX-M-2 +TEM-1 +SHV-12 + KPC-2	
KP30	B	2013		TEM-1 +SHV-12	
KP43	B	2013		TEM-1 +SHV-12 + KPC-2	
KP44	B	2013		TEM-1 +SHV-12	
KP50	B	2013		TEM-1 +SHV-12 + KPC-2	
KR02	D	2012		KPC-2	
KR03	D	2012		KPC-2	
KP36	B	2013		TEM-1 +SHV-12 + KPC-2	
KR17	D	2013	14	KPC-2	
KP10	B	2012		TEM-1 +KPC-2	
KR05	D	2013		KPC-2	
KR07	D	2013	14	NEGATIVE	
KR12	D	2013			
KP16	B	2012		KPC-3	
KL09	C	2012	512	CTX-M-15 +TEM-1 + KPC-2	X
KP59	B	2014		KPC-3	X
KH09	A	2013		KPC-3	X
KL53	C	2014		TEM-1 + KPC - 3	X
KL60	C	2014		KPC-3	X
KL56	C	2014		TEM-1 + KPC-2	X
KL33	C	2013	512	TEM-1 + KPC-3	X
KH28	A	2013		TEM-1 + KPC-3	X
KP23	B	2013	512	SHV-12 + KPC-3	X
KL15	C	2012	512	NEGATIVE	X
KL58	C	2014		KPC-3	X
KP58	B	2014	512	KPC-3	X
KL64	C	2014		KPC-3	X
KH01	A	2012		CTX-M-8 + KPC-3	X
KR11	D	2013		KPC-3	X
KP01	B	2012		KPC-3	X
KL18	C	2012		TEM-1 + KPC-3	X
KL24	C	2013		TEM-1 + KPC-3	X
KL30	C	2013		TEM-1 + KPC-3	X
KL21	C	2013		TEM-1 + KPC-3	X
KL27	C	2013	512	TEM-1 + KPC-3	X
KL51	C	2013		TEM-1 + KPC-3	X
KH44	A	2014		KPC-2	X
KP15	B	2012		SHV-12 + KPC-3	X
KP31	B	2013	11	KPC-2	
KH07	A	2012	268	CTX-M-15 + KPC-3	
KH38	A	2014	23	KPC-2	
KL48	C	2014	1703	KPC-2	
KR20	D	2014	283	CTX-M-15	
KP34	B	2013	40	KPC-3	
KP57	B	2014	1533	KPC-2	
KP11	B	2012	636	CTX-M-2 + TEM-1	
KH34	A	2013	280	CTX-M-15 + TEM-1	
KC03	E	2014	1705	SHV-12 + KPC-2	
KC06	E	2013	560	CTX-M-15 + TEM-1 + KPC-2	
KH11	A	2014	17	KPC-2	
KH03	A	2012	45	KPC-3	
KH30	A	2013		NEGATIVE	
KP06	B	2012		NEGATIVE	
KH20	A	2013	307	CTX-M-15 +TEM-1 + KPC-2	
KH35	A	2013		CTX-M-15 +TEM-1 + KPC-3	
KH23	A	2013		CTX-M-15 + TEM-11	
KH33	A	2013		CTX-M-15 +TEM-1 + KPC-2	
KH18	A	2013		CTX-M-15 +TEM-1 +KPC-2	
KH41	A	2014		CTX-M-15 +TEM-1 + KPC-2	
KH43	A	2014	307	CTX-M-15 +TEM-1 + KPC-3	
KH16	A	2013		CTX-M-15 +TEM-1 +SHV-12 + KPC-3	
KL57	C	2014		CTX-M-15 + TEM-1 + SHV-12 + KPC-3	
KH42	A	2014		CTX-M-15 +TEM-1 + KPC-3	
KH31	A	2013		CTX-M-15 +TEM-1 + KPC-3	
KH21	A	2013		CTX-M-15 +TEM-1 + KPC-2	
KL49	C	2013	307	CTX-M-15 +TEM-11 + KPC-2	
KH06	A	2012	1661	KPC-3	
KH8	A	2012	129	KPC-2	
KP19	B	2012	1704	KPC-2	
KH14	A	2013	151	CTX-M-15 +TEM-11 + KPC-2	
KH27	A	2013	971	KPC-2	
KP04	B	2013	147	VIM	
KL20	C	2013	35	TEM-1 + KPC-2	
KL36	C	2013	259	KPC-2	
KL37	C	2013	231	KPC-2	
KP56	B	2014	1887	KPC-2	

FIG 1 PFGE dendrogram generated with Bionumerics software showing the genetic relationship between representative strains of carbapenem-resistant *Klebsiella pneumoniae*. The analysis of the bands generated was performed by using the Dice similarity coefficient and the unweighted pair group method with arithmetic averages. The dashed line corresponds to 80% as the cutoff for close genetic relationship.

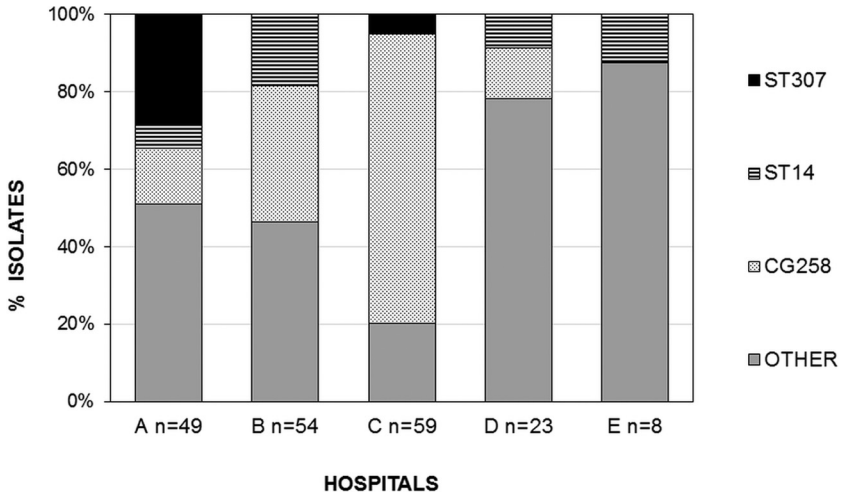


FIG 2 Relative distribution of major carbapenem-resistant *Klebsiella pneumoniae* clones found in this study—CG258, ST14, ST307, and other non-CG258 isolates—from five tertiary care hospitals (A to E) during the years 2012 to 2014 in Medellín, Colombia.

Isolates from the same CG or ST displayed highly similar PFGE profiles in each group (Fig. 1), further demonstrating their clonal dissemination. Our study indicates that both horizontal transfer and clonal spread have significantly contributed to the dissemination of carbapenem resistance in the area considered.

Among these CR-Kp clones, KPC-3-producing CG258, mainly ST512, was the most predominant, comprising 37.8% of the isolates, similar to observations in other studies reported previously in Colombia (14). CR-Kp CG258 appears to be the main clone in other Latin American countries where KPC is endemic, such as Argentina, Uruguay, and Brazil. Distinct to our study is the observation that the most prevalent CG258 STs, including ST258, ST11, and ST437, are mainly associated with KPC-2, suggesting that multiple CR-Kp CG258 sublineages are circulating in Latin America. ST512 was initially described in 2006 in hospital B, as the cause of the first outbreak of KPC-producing *K. pneumoniae* in Colombia (12, 13). Clearly, this clone has emerged in other institutions (hospitals A, C, and D). Meanwhile, the frequency of CG258 infections appeared to decrease over the study period (Fig. 3). Of clinical importance, isolates resistant to both tigecycline and colistin or colistin alone were predominantly found in this clone. Similar events have been described in countries where CG258 is highly prevalent (35–42), and in these areas where KPC-Kp is highly endemic and colistin exposure is high, resistance has been reported (38). This raises a significant concern given the fact that colistin is among the few agents that retain activity against CR-Kp and is the key component of the combination antimicrobial regimens that are frequently administered (2, 5, 36, 43, 44).

The second most common clone, ST14, has been regarded as a high-risk *K. pneumoniae* clone (45), known for its ability to accu-

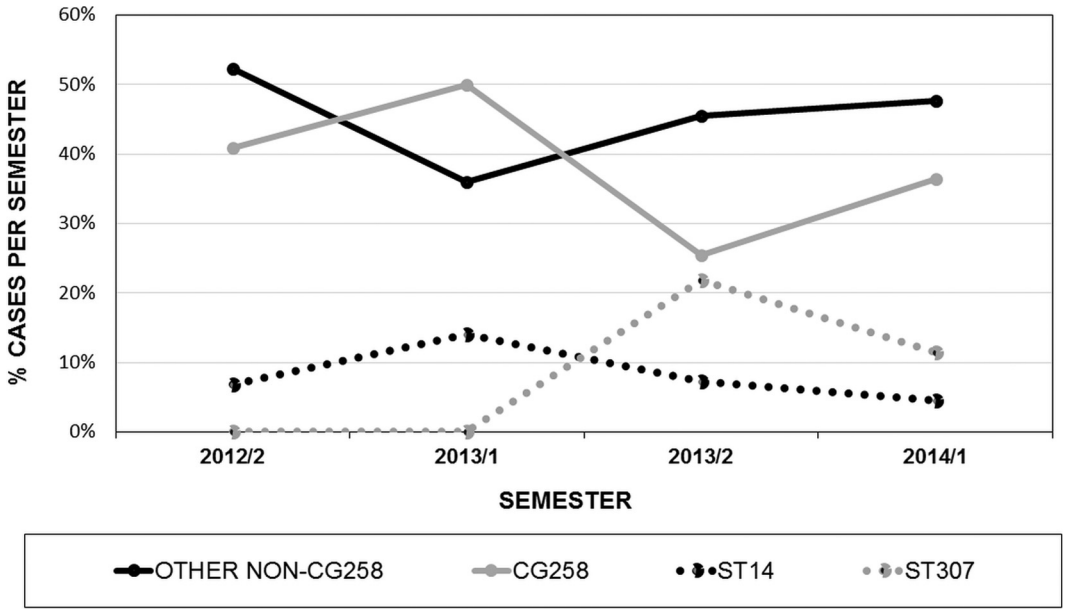


FIG 3 Relative distribution of cases per semester of major carbapenem-resistant *Klebsiella pneumoniae* clones found in this study.

mulate and to disseminate resistance determinants, including multiple β-lactamases, such as those in the KPC, NDM, CTX-M, SHV, TEM, FOX, CMY, and OXA families. ST14 has been reported in Italy (46), the United Kingdom, Sweden (47), Turkey (48), Spain (49), Tanzania (50), Kenya (51), India (47, 52), and Oman (53) and has been found harboring KPC in various facilities in the midwestern United States (54). In addition, it has been reported in sporadic cases carrying KPC-2, CTX-M-15, and TEM-1 in several cities of Colombia (55) and carrying KPC-2 and SHV-12 in Medellín (14). This is consistent with our results, where the profile TEM-1+SHV-12+KPC-2 was the most frequent. It is noteworthy that most patients infected with the ST14 strains were pediatric, which is likely associated with an ST14 CR-Kp outbreak in the pediatric ward in hospital B. However, ST14 has been identified in four out of five hospitals, demonstrating its wide spread in our area. Infections caused by carbapenem-resistant *Enterobacteriaceae* in infants and children are of great concern, as these individuals do not tolerate the various available agents, which frequently makes carbapenems the last-resort therapeutic option for treating severe infections in children. Curiously, reports of pediatric outbreaks caused by ST14 were found in Italy, where isolates carried FOX-7 AmpC β-lactamase (46); in Tanzania, where isolates carried the ESBL CTX-M-15 (50); and in Turkey, where isolates carried NDM-1 (48).

ST307, the third most common clone in this study, has not been described in Colombia, and this is the first report of this clone in our country. Most significantly, the mortality associated with ST307 infections is over 50%. Further investigations are needed to assess if this clone is associated with hypervirulence and carries virulence genes. On the other hand, ST307 strains emerged in the second half of 2013, were predominately identified in hospital A, and displayed close PFGE profiles, suggesting an outbreak. The origin of the CR-Kp ST307 in this study remains unclear. It is possible that a carbapenem-susceptible strain acquired a KPC plasmid from another strain (e.g., CG258) or that an ST307 strain was imported from somewhere else. Interestingly, this clone has been reported to harbor KPC-2 and KPC-3 in the United States (56) and in Italy (57), where it seems to be replacing ST258 (58, 59). In addition, it has been reported to carry OXA-48 and CTX-M-15 in Morocco (60), which coincides with our results showing that 94.1% of ST307 isolates carried this ESBL.

In general, the clinical features of CR-Kp infection were in agreement with previous studies. For example, our findings are consistent with the observations in previous studies that described most patients to be elderly, with multiple comorbidities (2, 57, 61) and with UTI and bloodstream infections the most frequently reported (42, 57, 61–63). Several studies evaluating risk factors for CR-Kp acquisition found exposure to health care and previous antimicrobial use as the most significant risks (3, 9, 61–69). This is consistent with our findings that medical history showing surgery, hospitalization, antibiotic use prior to infection, and the presence of invasive medical devices at the moment of infection were associated with CR-Kp acquisition (Table 1). Meanwhile, we found an overall 34% mortality rate, in accordance with previous reports of rates that varied from 6% to 60% depending on the severity of infection (9, 42, 68–70). Interestingly, our study revealed a highly heterogeneous genetic background of CR-Kp strains, and CR-Kp from different clones displayed distinct clinical characteristics in our local epidemiology. Although the factors contributing to the clinical differentiation of CR-Kp remain unclear, multiple factors, including the various selective pressures, unique genetic contents, and different host responses, may play a role.

Taken together, the results in this study describe the heterogeneous spread of carbapenem resistance in a region where KPC is endemic. Remarkably, non-CG258 strains account for >60% of CR-Kp isolates, highlighting the significant contribution of horizontal transfer in the spread of carbapenem resistance. More interestingly, the non-CG258 strains demonstrated distinct clinical characteristics in comparison to CG258, suggesting that they may adapt to different ecological niches other than CG258 strains, further complicating clinical treatment and infection control strategies. In addition, our study evidenced the strong benefits of real-time surveillance and use of molecular biology to support epidemiological research in tracking the emergence and dissemination of resistance clones and examining the clinical characteristics of patients infected by such clones, which may provide useful data to guide resistance and infection control strategies.

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